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Early prenatal diagnosis of recurrent 46,XY partial gonadal dysgenesis

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Objectives We present a case of early prenatal diagnosis of recurrent 46,XY partial gonadal dysgenesis, by combining early genetic and sonographic evaluations.

Methods The conceptus of a mother with a first child affected by 46,XY gonadal dysgenesis was sonographically evaluated at 21- and 23-mm BPD (12^{+2} and 12^{+6} LMP-based age) and the female genitalia were observed. Karyotype analyses was performed on amniotic fluid and it revealed a 46,XY complement without mosaicism. SRY was amplified by PCR for molecular analyses.

Results We observed a discordance between female phenotype detected at 21 and 23 mm of biparietal diameter (12^{+2} and 12^{+6} LMP-based age) and male karyotype. In the child and the fetus, seminiferous cords were not recognisable, whereas rare Leydig cells and no germ cells could be identified. Internal and external genitalia were sexually ambiguous in the child and feminized in the fetus.

Conclusion This is the first case of early prenatal diagnosis of recurrent 46,XY partial gonadal dysgenesis and it points to the importance of combining early analyses of genetic sex with sonography in the management of anomalies of sexual development, with particular regard to syndromes for which the risk of recurrence is little understood. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: XY partial gonadal dysgenesis; recurrence; fetal gender; prenatal diagnosis; sonography

INTRODUCTION

Sexual differentiation in the eutherian mammal is a sequential process beginning with the establishment of chromosomal sex at fertilization, followed by the development of gonadal sex, and culminating in the formation of the sexual phenotypes (George and Wilson, 1994). Each step in this process is dependent on the preceding one, and under normal circumstances, chromosomal sex agrees with phenotypic sex. Occasionally, however, chromosomal sex and phenotypic sex do not agree, and the sexual phenotype is ambiguous.

Sonographic fetal-gender determination has become an important part of routine prenatal examination. Accuracy of sonography in early pregnancies is now well estabilished.

There have been a number of studies using ultrasound to detect and correctly identify fetal gender in the early second and third trimester (Stephens and Sherman, 1984), (Birnholz, 1983), (Plattner *et al*., 1983). As a consequence of improvement in ultrasound technology, and with the advent of transvaginal sonography, some authors have begun to use it in the first trimester itself.

In Whitlow's work, the overall success of correctly identifying fetal gender increased with gestation from 46 to 75 to 79% and then to 90% at 11, 12, 13, and 14 weeks respectively (confirmed by crown-rump length (CRL)); the overall success of correctly identifying the fetal gender at 12 to 14 weeks was found to be 80% (Whitlow *et al*., 1999). According to Benoit, fetal gender was correctly identified in 98.5% at 12 weeks and in 100% at 13 weeks (Benoit, 1999).

According to Mazza, the accuracy of fetal-sex prediction increased from 60% at 18 mm of biparietal diameter (BPD) to 100% at BPD of 23 mm (corresponding to 12^{+6} LMP-based age) (Mazza *et al*., 1999).

According to Efrat, the accuracy of sex determination increased with gestation from 70.3% at 11 weeks to 98.7% at 12 weeks and to 100% at 13 weeks (corresponding to 68 mm of CRL) (Efrat *et al*., 1999).

With advances in technology, it has now become possible to detect structural genital anomalies (Mandell *et al*., 1995), and, recently, accuracy in the prenatal diagnosis of abnormal genitalia and its impact on post-natal management was retrospectively analysed (Cheikhelard *et al*., 2000). In routine circumstances, when the sonographic and the cytogenetic findings show that sex is discordant, a gonadal or genital developmental anomaly is suspected. In 1984, Stephens reported the first prenatal diagnosis of testicular feminization discovered by a difference between a sonographically female phenotype and a 46,XY karyotype (Stephens, 1984). Recently, we have described an early prenatal diagnosis

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of female pseudohermaphroditism associated with bilateral luteoma of pregnancies on the basis of male phenotype revealed by sonography at 23-mm BPD (12^{+6} last menstrual period (LMP)-based age) and normal female karyotype (Mazza *et al*., 2002).

Here, we present a case of early prenatal diagnosis of recurrent 46,XY partial gonadal dysgenesis on the basis of discordance between sonographic fetal-sex determination and karyotype.

CASE REPORT

A woman, gravida 2 para 1, was referred to our prenatal unit at 12^{+2} LMP-based age because the first child was affected by 46,XY partial gonadal dysgenesis.

The pedigree of the family did not reveal significant genital anomalies (only the paternal uncle of the proband's father was affected by bilateral cryptorchidism).

At birth her first child presented a phallus of 1.5 cm, perineoscrotal hypospadias, a blind vagina, and a bifid scrotum. The karyotype was 46,XY without mosaicism. Therefore, he was assigned and raised as a male.

A hypoplastic uterus with bilateral ducts resembling tubes was reported at laparotomy (aged three years), while intra-abdominal gonads appeared anatomically to be the testes. Bilateral gonadectomy was performed at the age of four years. The testes contained dysgenetic seminiferous cords devoid of germ cells with rare Leydig cells. The histology was consistent with the differentiation of a vas deferens on the left side, whereas the presence of a hypotrophic tube was confirmed on the right side. Masculinizing genitoplasty was completed at the age of six years. No extragenital anomalies were observed at birth and on follow-up (presently the child is 10 years old).

The karyotype was 46,XY without mosaicism.

Gonadotrophins and testosterone were measured at two months of age and appeared in the normal range for a prepubertal male [FSH = 4.2 mU/mL (2–9.8); LH < 0.5 mU/mL (0.5–6.9); T = 0.17 ng/mL (0–20)]. A response of testosterone production to human chorionic gonadotrophins could be detected (from 0.01–0.42 ng/mL).

Molecular analyses included a study of *SRY*, which was amplified by PCR on the DNA from the patient, and the androgen receptor. No mutations were found by DNA sequencing in the androgen receptor gene, and no quantitative anomalies of binding capacity (reflecting the number of expressed receptors) or affinity for the ligand were detected in cultured skin fibroblasts [Scatchard analysis: B_{max} = 689 fmol/mg DNA (659–967); K_d = 0.29 nmol/L (0.23–0.25)]. Finally, analysis of DNA sequences harbouring known single-nucleotide polymorphisms from distal 9p showed that the patient had two different alleles in this region, thereby excluding large deletions (Ottolenghi *et al*., 2000).

In the present pregnancy, the fetus was first sonographically evaluated at 21-mm BPD (12^{+2} LMP-based age). The genitalia appeared to be female (Figure 1A), but in our experience, sonographic sexing at this age is not fully specific. Therefore, a second sonography was performed at 23-mm BPD (12^{+6} LMP-based age) and female genitalia were confirmed. At this age, our experience shows that sonography is fully discriminative between sexes (Mazza *et al*., 1999). Two additional sonographic evaluations were performed as part of the follow-up of the pregnancy, at 30- and 41-mm BPD (Figures 1B–1D), confirming the previous sex assignment of genitalia. Karyotype analysis was performed on amniotic fluid and it revealed a 46,XY complement without mosaicism. Pregnancy was interrupted at 17 weeks in another hospital for psychosocial reasons.

The fetus had well-developed female external genitalia, with unfused labia majora and hypoplastic labia minora (Figure 2), as usually observed before 20 weeks (Ammini *et al*., 1994). Internal genitalia were represented by well-developed müllerian derivatives (bilateral tubes and uterus with normal cytodifferentiation) (Figure 3A). Some mesonephric tubular structures lined by cuboidal cells were located adjacent to the gonads (Figure 4A), as is commonly found in both sexes (Figures 4B and 4C). We failed to detect well-developed wolffian derivatives. Extragenital anomalies, particularly growth delay, anomalies of long bones, and renal anomalies, were not observed. Both gonads were dysgenetic and had similar appearance. First, they had an elongated shape and lacked a tunica albuginea (Figures 3 and 4D). These features are reminiscent of indifferent gonads, and can be distinguished from fetal ovaries by the absence of small cuboidal cells characteristic of the ovary surface epithelium, as shown for a control ovary at 13 weeks (Figure 4E). In addition, no follicles or germ cells of either sex were observed. Second, neither gonad contained recognisable seminiferous cords, and only rare, small clusters of Leydig-like cells could be detected (large, polygonal cells with eosinophilic cytoplasm and amphiphilic nuclei with prominent nucleoli). The latter contrasts with the usual peak of abundance of Leydig cells in the human fetal testis at 17 to 19 weeks (Waters and Trainer, 1996) (and Figure 4F).

These data indicate that both the child and the fetus were affected by 46,XY partial gonadal dysgenesis.

DISCUSSION

Approximately 1 in 5000 babies is born with ambiguous genitalia (Kutteh *et al*., 1995). The classification of this disorder in newborns is difficult because similar phenotypes can have several different aetiologies. In most cases, it was not possible to correlate aetiology of the disorder with the degree of anomalies of the external genitalia. In many cases, disorders of sexual differentiation are inherited as single-gene mutations, and their analysis has been especially informative in defining the molecular and genetic determinants in sexual development and risk of recurrence (Sarafoglou and Ostrer, 2000).

The term *gonadal dysgenesis* is generally used to describe a variety of clinical conditions in which

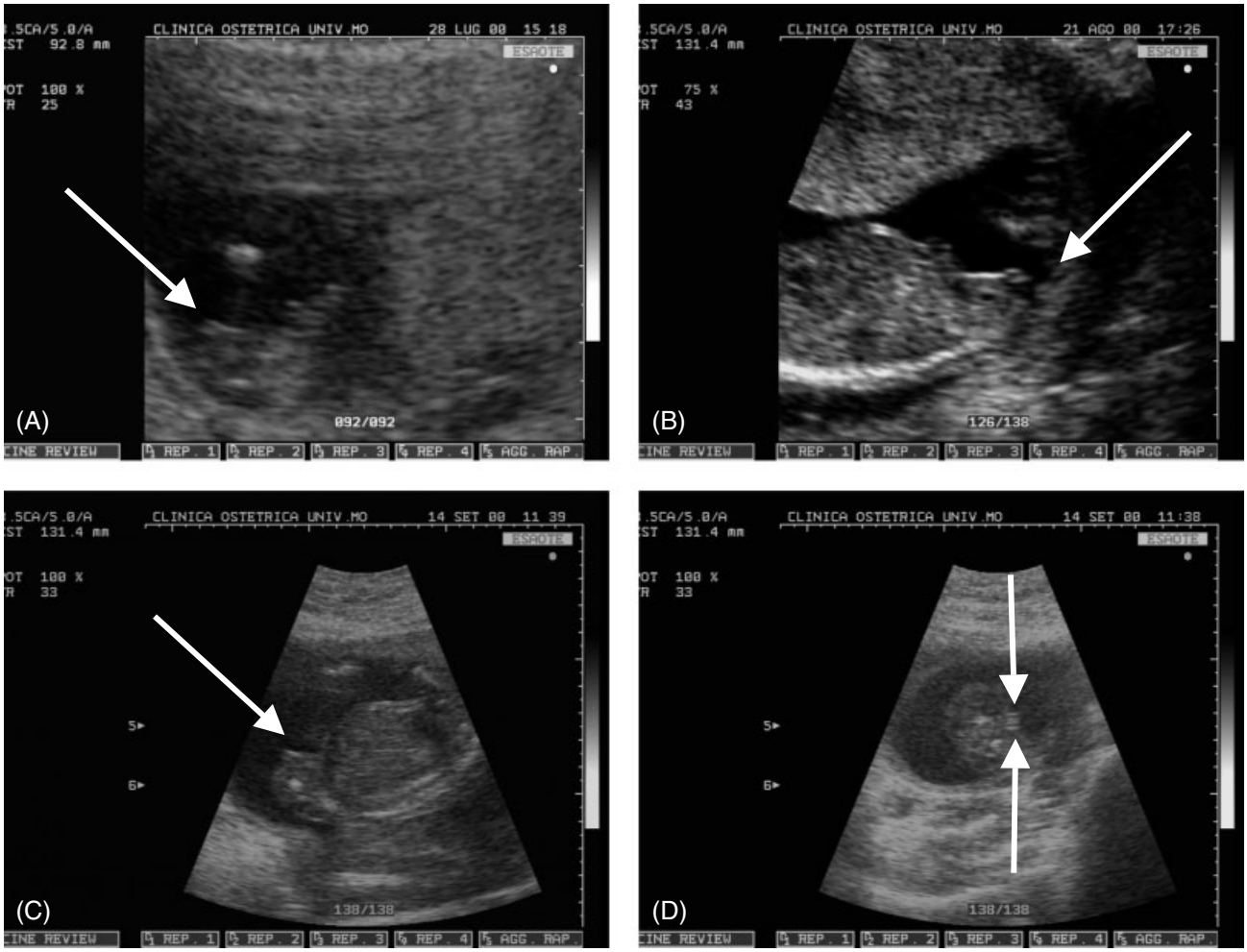

Figure 1—Sonography for fetal-gender assignment. (A, B, C) Sagittal sections of external genitalia at 21-, 30- and 41-mm BPD, respectively. The arrows point to the bulge/clitoris. (D) Coronal section of external genitalia at 41-mm BPD. The arrows point to the labia majora and minora

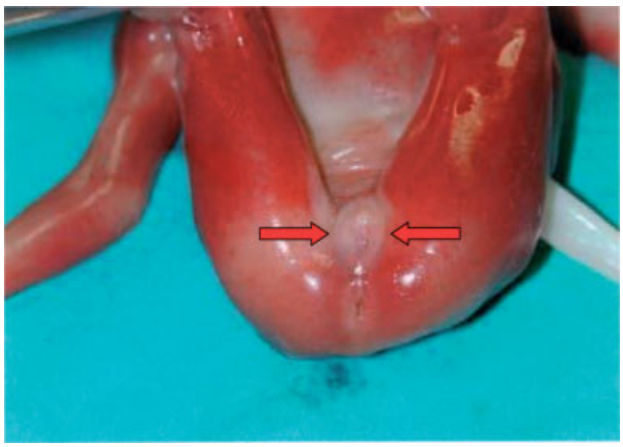

Figure 2—External genitalia of the fetus with 46,XY partial gonadal dysgenesis at 17 weeks' gestation. The arrows point to the unfused labia majora

development of the fetal gonad is abnormal in the presence of either normal sex karyotype, 45,X karyotype, or 45,X/46,XY mosaicism. According to a widely followed classification, the condition 46,XY gonadal dysgenesis comprises two forms, termed *complete* and *partial*.

(Berkovitz *et al*., 1991). Complete 46,XY gonadal dysgenesis, also termed *pure* to indicate the absence of Turner stigmata, is associated with complete phenotypic male-to-female sex reversal and with streak gonads. The term *partial 46,XY gonadal dysgenesis* is used whenever ambiguous or female genitalia are observed in the presence of abnormal testicular differentiation, that is, whenever seminiferous tubules are poorly developed, or partly replaced by ovarian-like stroma, in one or both gonads. Partial 46,XY gonadal dysgenesis is sometimes referred to as *46,XY mixed gonadal dysgenesis* and *dysgenetic male pseudohermaphroditism* (Berkovitz *et al*., 1991; Berkovitz, 1992).

Interestingly, in the case presented here, a severe form of gonadal dysgenesis is described in a fetus aged 17 weeks. The absence of recognisable seminiferous cords and the development of müllerian derivatives, which are fated to regress by about 12 weeks in human male embryos (Jost, 1972), indicate that the gonads were non-functional before, or at the time of, gonadal sex determination; that is, müllerian inhibiting substance

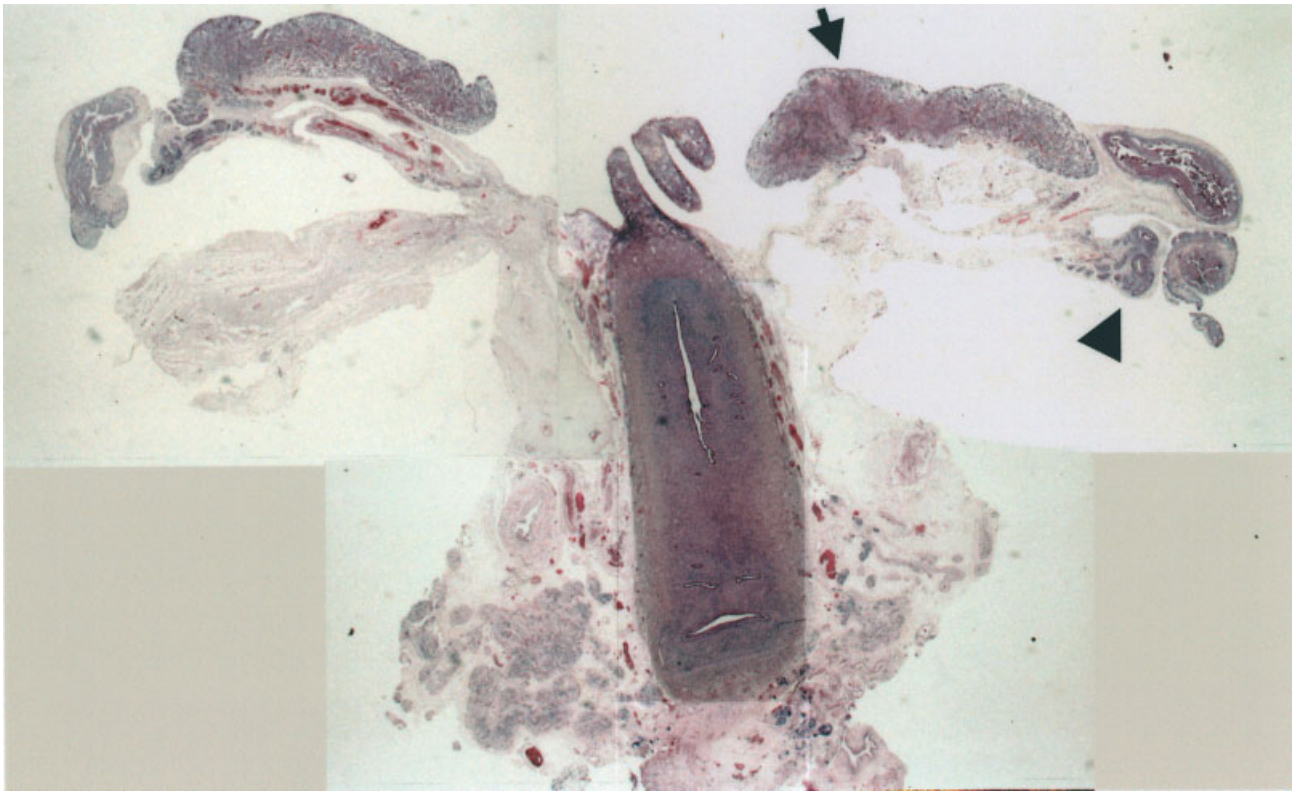

Figure 3—Histology of the gonads and internal genitalia of the affected fetus aged 17 weeks (hematoxylin–eosin staining, HES; ×1.25). Arrowhead and arrow: portion of the mesonephric derivatives and portion of the gonad, which are shown with greater magnification in Figures 4A and 4D, respectively

anti-müllerian hormone (MIS/AMH) was not produced to any significant extent. Similarly, female development of internal and external genitalia suggests early impairment of the function of the male sex steroid-secreting (Leydig) cells. Consistent with these results, Leydig cells were very rare in both gonads (Figures 4A and 4D), contrasting with the features of age-matched testes as shown in Figure 4E. Incidentally, the sequence and function of the androgen receptor gene, although not a likely candidate for gonadal anomalies (Chen *et al*., 1999) was shown to be normal in the child from the previous pregnancy. Combined with the ovary-like shape of the gonads and the absence of tunica albuginea resembling indifferent gonads (compared with the ovary of a younger fetus, Figure 4F), these data suggest that testis differentiation, although initiated to some extent, was severely abnormal at a very early stage.

While partial masculinization of internal and external genitalia indicates that the underlying mutation was less penetrant in the child from the previous pregnancy than in the fetus, it is remarkable that the former had similar gonadal anomalies, particularly an immature appearance, no germ cells, and scattered Leydig cells.

Both the child and the fetus had no extragenital anomalies. The aetiology of non-syndromic forms of 46,XY partial gonadal dysgenesis is still poorly understood. The gene *SRY* is rarely mutated in the partial form, and most other chromosomal rearrangements and intragenic mutations are systematically associated with extragenital anomalies (Bardoni *et al*., 1994; Nordenskjold *et al*., 1995; Kwok *et al*., 1996). A region on distal 9p is known to be deleted in several patients with isolated 46,XY gonadal dysgenesis (Bennett *et al*., 1993; and references therein), but in our case, analysis of nucleotide polymorphisms excluded the presence of a large deletion of the region.

46,XY partial gonadal dysgenesis could arise from a partial defect in the testis-determining factor (TDF) function or in another gene involved in the early stages of testicular differentiation. Although TDF is the trigger, a variety of other genes must also be involved in this differentiation, but few candidates have been identified (Berkovitz *et al*., 1991). Studies of mice also suggest a role for autosomal genes in testicular determination (Washburn and Eicher, 1983).

In addition, there is an X-linked form of 46,XY gonadal dysgenesis.

Both the complete and partial forms of 46,XY gonadal dysgenesis are usually sporadic, but familiarity, which is mostly limited to sibships, has been ascertained in some cases.

Here, we present one of the rare cases of recurrent familial 46,XY partial gonadal dysgenesis described in literature (Fechner *et al*., 1993), (Phansey *et al*., 1980), (Barr *et al*., 1967).

Incompletely penetrant *SRY* mutations have been reported in some families with 46,XY complete gonadal dysgenesis, whilst in other cases, inheritance can be X-linked, autosomal recessive, or sex-limited autosomal dominant (Espiner *et al*., 1970; German *et al*., 1978;

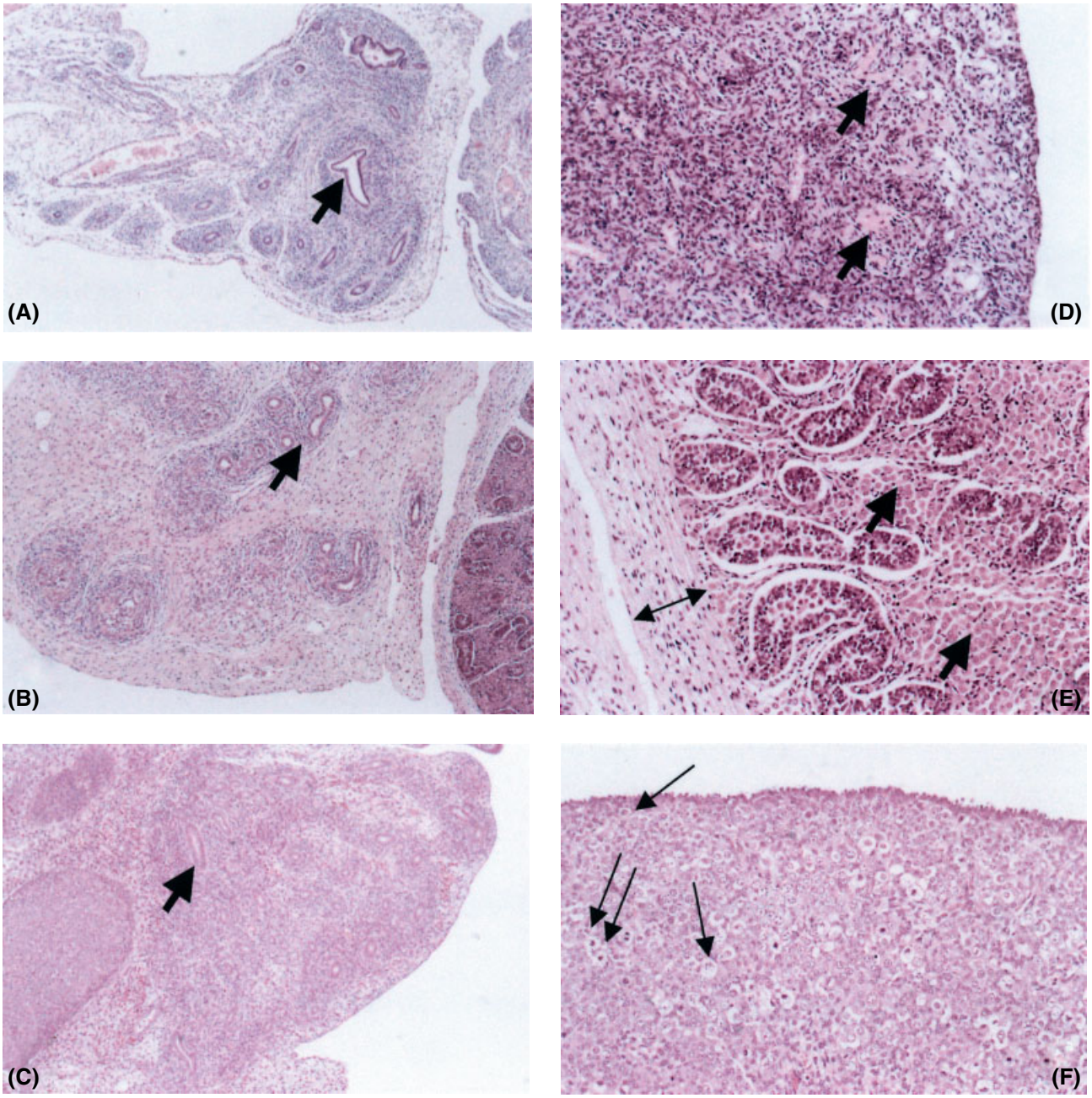

Figure 4—Histology of the hilar portion of the gonads (HES; x8), including mesonephric tubules (A, B, C), and details of the gonads (HES; ×20) (D, E, F). (A, D) Dysgenetic gonads–affected fetus aged 17 weeks; (B, E) testes from a normal male fetus aged 18 weeks; and (C, F) ovaries from a normal female fetus aged 13 weeks. In the left panel, the arrows indicate mesonephric tubules. In the right panel, the thick arrows indicate Leydig cells, the thin arrows indicate germ cells, and the bidirectional arrow spans the thick tunica albuginea

Mann *et al*., 1983; Boczkowski, 1976; Naffah, 1989; Mendonca *et al*., 1994; Kempe *et al*., 2002; Josso and Briard, 1980; Calvari *et al*., 2000; Barr *et al*., 1967; Phansey *et al*., 1980; Fechner *et al*., 1993; Acquafredda *et al*., 1987). Although the incidence of recurrent cases is not known, our report draws attention to the fact that following the diagnosis of a case of 46,XY partial gonadal dysgenesis, close scrutiny of subsequent pregnancies by combining karyotype analysis and sonography is highly informative and should be performed.

With recurrent fetal pathologies, in particular, those related to genital anomalies, parents often have opposite decisional reactions in case of a second pregnancy with an affected fetus, even when it is possible to treat with appropriate means and to enable a good quality of life.

In our case, we proposed the couple the assignment at birth of female sex to the second child, even though the karyotype was 46,XY; nevertheless, they decided to terminate the pregnancy.

However, we believe that genetic counselling will benefit from obtaining precise and early information about whether sexual development of a fetus is abnormal.

The choice of therapeutic abortion, as decided in our family, can be debated for cases of isolated 46,XY gonadal dysgenesis.

It should be stressed that the modes of transmission that can be inferred from the familial cases described so far (Fechner *et al*., 1993), (Phansey *et al*., 1980), (Barr *et al*., 1967) suggest that the risk of recurrence for karyotypic males in cases of 46,XY partial gonadal dysgenesis should be evaluated by assuming Mendelian inheritance.

In conclusion, in our experience, the sex of external genitalia can be assessed with full discrimination at 23-mm BPD (12^{+6} LMP-based age) by sonography. This permits early recognition of a discordance between genetic sex, as established by karyotype and analysis of *SRY*, and phenotypic sex, based on sonographic evaluation of external genitalia. Combining early analyses of genetic and phenotypic sex will be instrumental for the diagnosis of familial anomalies of sexual development, as in this case, which represents the first report of early prenatal diagnosis of recurrent 46,XY partial gonadal dysgenesis.

REFERENCES

Acquafredda A, Vassal J, Job JC. 1987. Rudimentary testes syndrome revisited. *Pediatrics* **80**: 209–214.

Ammini AC, Pandey J, Vijayaraghavan M, Sabherwal U. 1994. Human female phenotypic development: role of fetal ovaries. *J Clin Endocrinol Metab* **79**: 604–608.

Bardoni B, Zanaria E, Guioli S, *et al*. 1994. A dosage sensitive locus at chromosome Xp 21 is involved in male to female sex reversal. *Nat Genet* **7**: 497–501.

Barr ML, Carr DH, Plunkett ER, Soltan HC, Wiens RG. 1967. Male pseudohermaphroditism and pure gonadal dysgenesis in sisters. *Am J Obstet Gynecol* **99**: 1047–1055.

Bennett CP, Docherty Z, Robb SA, Ramani P, Hawkins JR, Grant D. 1993. Deletion 9p and sex reversal. *J Med Genet* **30**(6): 518–520.

Benoit B. 1999. Early fetal gender determination. *Ultrasound Obstet Gynecol* **13**: 299–300.

Berkovitz GD, Fechner PY, Zacur HW, *et al*. 1991. Clinical and pathologic spectrum of 46,XY gonadal dysgenesis: its relevance to the understanding of sex differentiation. *Medicine (Baltimore)* **70**: 375–383.

Berkovitz GD. 1992. Abnormalities of gonadal determination and differentiation. *Semin Perinatol* **16**: 289–298.

Birnholz JC. 1983. Determination of fetal sex. *N Engl J Med* **309**: 942–944.

Boczkowski K. 1976. Familial occurrence of gonadal tumors in XY females with breast development. *Hum Genet* **33**: 289–294.

Calvari V, Bertini V, De Grandi A, *et al*. 2000. A submicroscopic deletion that refines the 9p region for sex reversal. *Genomics* **65**: 203–212.

Cheikhelard A, Luton D, Philippe-Chomette P, *et al*. 2000. How accurate is the prenatal diagnosis of abnormal genitalia? *J Urol* **164**: 984–987.

Chen CP, Chen SR, Wang TY, Wang W, Hwu YM. 1999. A frame shift mutation in the DNA-binding domain of the androgen receptor gene associated with complete androgen insensitivity, persistent mullerian structures, and germ cell tumors in dysgenetic gonads. *Fertil Steril* **72**: 170–173.

Efrat Z, Akinfenwa OO, Nicolaides KH. 1999. First-trimester determination of fetal gender by ultrasound. *Ultrasound Obstet Gynecol* **13**: 305–307.

Espiner EA, Veale AM, Sands VE, Fitzgerald PH. 1970. Familial syndrome of streak gonads and normal male karyotype in five phenotypic females. *N Engl J Med* **283**: 6–11.

Fechner PY, Marcantonio SM, Ogata T, *et al*. 1993. Report of a kindred with X-linked (or autosomal dominant sex-limited) 46,XY partial gonadal dysgenesis. *J Clin Endocrinol Metab* **76**: 1248–1253.

George FW, Wilson JD. 1994. Sex determination and differentiation. In *The Physiology of Reproduction*, Knobil E, Neill JD (eds). Raven Press: New York.

German J, Simpson JL, Chaganti RS, Summitt RL, Reid LB, Merkatz IR. 1978. Genetically determined sex-reversal in 46,XY humans. *Science* **202**: 53–56.

Josso N, Briard ML. 1980. Embryonic testicular regression syndrome: variable phenotypic expression in siblings. *J Pediatr* **97**: 200–204.

Jost A. 1972. A new look at the mechanisms controlling sex differentiation in mammals. *Johns Hopkins Med J* **130**: 38–53.

Kempe A, Engels H, Schubert R, *et al*. 2002. Familial ovarian dysgerminomas (Swyer syndrome) in females associated with 46 XY-karyotype. *Gynecol Endocrinol* **16**: 107–111.

Kutteh WH, Santos-Ramos R, Ermel LD. 1995. Accuracy of ultrasonic detection of the uterus in normal newborn infants: implications for infants with ambiguous genitalia. *Ultrasound Obstet Gynecol* **5**: 109–113.

Kwok C, Goodfellow PN, Hawkins JR. 1996. Evidence to exclude SOX9 as a candidate gene for XY sex reversal without skeletal malformation. *J Med Genet* **33**: 800–801.

Mandell J, Bromley B, Peters CA, Benacerraf BR. 1995. Prenatal sonographic detection of genital malformations. *J Urol* **153**: 1994–1996.

Mann JR, Corkery JJ, Fisher HJ, *et al*. 1983. The X linked recessive form of XY gonadal dysgenesis with a high incidence of gonadal germ cell tumours: clinical and genetic studies. *J Med Genet* **20**: 264–270.

Mazza V, Contu G, Falcinelli C, *et al*. 1999. Biometrical threshold of biparietal diameter for certain fetal sex assignment by ultrasound. *Ultrasound Obstet Gynecol* **13**: 308–311.

Mazza V, Di Monte I, Ceccarelli PL, *et al*. 2002. Prenatal diagnosis of female pseudohermaphroditism associated with bilateral luteoma of pregnancy: case report. *Hum Reprod* **17**: 821–824.

Mendonca BB, Barbosa AS, Arnhold IJ, McElreavey K, Fellous M, Moreira-Filho CA. 1994. Gonadal agenesis in XX and XY sisters: evidence for the involvement of an autosomal gene. *Am J Med Genet* **52**: 39–43.

Naffah J. 1989. Familial testicular regression syndrome. *Bull Acad Natl Med* **173**: 709–714; discussion 714–715.

Nordenskjold A, Fricke G, Anvret M. 1995. Absence of mutations in the WT1 gene in patients with XY gonadal dysgenesis. *Hum Genet* **96**: 102–104.

Ottolenghi C, Veitia R, Quintana-Murci L, *et al*. 2000. The region an 9p associated with 46,XY sex reversal contains several transcripts expressed in the urogenital system and a novel doublesex-related domain. *Genomics* **64**: 170–178.

Phansey SA, Satterfield R, Jorgenson RJ, *et al*. 1980. XY gonadal dysgenesis in three siblings. *Am J Obstet Gynecol* **138**: 133–138.

Plattner G, Renner W, Went J, Beudette L, Viau G. 1983. Fetal sex determination by ultrasound scan in the second and third trimesters. *Obstet Gynecol* **61**: 454–458.

Sarafoglou K, Ostrer H. 2000. Clinical review 111: familial sex reversal: a review. *J Clin Endocrinol Metab* **85**: 483–493.

Stephens JD. 1984. Prenatal diagnosis of testicular feminisation. *Lancet* **2**: 1038.

Stephens JD, Sherman S. 1984. Determination of fetal sex by ultrasonography. *N Engl J Med* **309**: 984.

Washburn LL, Eicher EM. 1983. Sex reversal in XY caused by dominant mutation on chromosome 17. *Nature* **303**: 338–340.

Waters BL, Trainer TD. 1996. Development of the human fetal testis. *Pediatr Pathol Lab Med* **16**: 9–23.

Whitlow BJ, Lazanakis MS, Economides DL. 1999. The sonographic identification of fetal gender from 11 to 14 weeks of gestation. *Ultrasound Obstet Gynecol* **13**: 301–304.